Macleay Bacteriologist.

During the year 1954–55 Council again granted permission for Dr. Yao-tseng Tchan to deliver lectures to advanced students in Agricultural Microbiology at the University of Sydney. In February, 1955, Dr. Tchan transferred from the Department of Botany to the Microbiological section of the Faculty of Agriculture of the University of Sydney, and permission was granted by the Council for him to do part-time work in that Faculty as from the middle of February, 1955. His work on the semi-arid soil is partly finished. Two papers, in collaboration with Professor N. C. W. Beadle, are ready for publication. The general conclusion of the results is that the amount of N fixed through bacteria and algae is small—a maximum of 2–3 pounds per acre per annum. However, if no loss occurs, it would be significant over long periods. The chemical analysis and bioassays of these soils (about 50 samples tested) showed that the limiting factor of plant growth in these soils is N if the water supply is non-limiting. No significant deficiency of P or Cu is recorded. It is logical for future studies in this region to concentrate on the N-economy of the soil through other biological processes (e.g. symbiotic N-fixation, denitrification and soil respiration). The study of a new technique of estimating soil protozoa with J. Burit is finished. A preliminary note has been published in *Nature*. The detailed paper will shortly be submitted for publication. Cytological investigations of *Azotobacter* have been continued. For his future research programme Dr. Tchan proposes to continue investigations on semi-arid soils and on soils of the Northern Territory, the latter in collaboration with C.S.I.R.O. (Land Research and Survey Section). More detailed work will be done on the estimation of soil fertility by microorganisms, and cytological and ecological investigations on N-fixing bacteria.

Obituaries.

It is recorded with regret that the following members and honorary member died during the year:

Mr. Kenneth George Brown, B.Sc., who was elected a member of the Society on 26th April, 1950, died on 7th April, 1954. On 30th July, 1952, he delivered a lecturette to the Society, illustrated by a film and exhibits, on the Natural History of Heard Island. He was biologist to the Australian Antarctic Expedition, 1951.

Mr. Wilfred Alexander Watt de Beuzeville, J.P., of Beecroft, N.S.W., who died on 28th March, 1954, had been a member of the Society since 1925. He contributed one paper (with Mr. C. T. White) to the Society's Proceedings in 1946. He was born at Bombala, N.S.W., where his father managed a group of stations, in 1884. In 1911 he joined the staff of the N.S.W. Forestry Department and was engaged on forest survey and assessment work until 1919, when he was placed in charge of the Forestry District extending approximately from Goulburn to the Victorian border, with headquarters at Tumut. During the next ten years he organized the establishment of about eight or ten large pine plantations in the district, in addition to the development of the natural forests in the area, especially the Alpine Ash forests at Batlow. From about 1930 to 1935 his services were made available to the C.S.I.R. Forest Products Division, and he collected botanical data from all parts of the eastern States. During the balance of his service with the Commission he was engaged in research work and established the experimental forest and nursery at Pennant Hills. It was during this period that he compiled his Climatic Index of the World, which required the analysis of rainfall and temperature statistics from each country. During the last years of his service as Forest Ecologist he wrote a number of pamphlets and finally the book "Australian Trees for Australian Planting", which was published in 1953. For more than forty years Mr. de Beuzeville preached the doctrine of soil conservation, and in his later years carried out a number of investigations for the Department, especially in the far west of the State. He was always a keen botanist and for many years corresponded regularly with the late J. H. Maiden. He described several new species of Eucalypts, sometimes working in collaboration with his friend the late M. B. Welch. Following his retirement Mr. de Beuzeville spent about a year in England and on the Continent,

returning to Sydney in 1951. During the next twelve months he was employed by the Food and Agriculture Organization of the United Nations, selecting lists of Australian trees suitable for reafforestation in Ethiopia and Syria. In February, 1953, he suffered a severe heart attack which left him an invalid until his death.

Mr. Ernest Godfried Jacobs, who had been a member of the Society since 1917, died on 10th July, 1954. For a number of years he was a regular attendant at monthly meetings. Mr. Jacobs was Headmaster of Christ Church Schools (Pitt Street, Sydney) for thirty-three years. He completed a course of training at Sydney Technical College for the Associate Diploma in Biology, which he gained, with honours, in February, 1912. During the absence in 1913–14 of Dr. S. J. Johnston in Europe he was Temporary Assistant Teacher of Botany at the Sydney Technical College, and in 1917 and 1918 was Permanent Assistant Teacher of Botany. Mr. Jacobs was always an interested and active member of the Workers' Educational Association Ramblers' and Naturalists' Club and for a number of years prior to his death was their president. Members of this Club planted a tree (*Melaleuca leucadendron*) to his memory at the W.E.A. Summer School, Newport, N.S.W., in November, 1954. His knowledge and skill in botany and allied sciences had gained the highest respect of all who knew him. He was a regular churchman and keenly interested in theology.

An Honorary Member of the Society since 1923, Professor James Peter Hill, D.Sc., F.R.S., died suddenly at his home in London on 24th May, 1954, in his eighty-first year. He came from Edinburgh to become demonstrator in biology at the University of Sydney in 1892, and in 1904 was lecturer in embryology. Professor Hill was a member of the Society from 1893 and a member of Council from 1901–1906. He published eleven papers in the Society's Proceedings between 1893 and 1900, including one as joint author with Professor W. A. Haswell and one with Professor C. J. Martin. He returned to England in 1906 and was Professor of Embryology at London University from 1921 to 1938, when he retired at the age of 65. He was elected to the Royal Society in 1913 and awarded the Darwin Medal in 1940. A more detailed account of his career, work and personality is given in *Nature*, Vol. 174, No. 4429, p. 109, July 17, 1954.

The Water Relations of Plant Cells.

(Plate A; Text-figures 1–3.)

I have chosen as the topic for the Presidential Address "The Water Relations of Plant Cells". I have made this choice for several reasons. First, because at the present time it is one of the controversial issues in botany; secondly, because drought is one of the major factors controlling the development of agriculture in Australia; and thirdly, because it is a subject which interests various members of the staff of the Botany Department at the University.

For approximately the last seventy years botanists have believed that osmosis was the mechanism underlying the water relations of plant cells. Before 1880 the process controlling the water relations of cells was only vaguely understood. Within ten years following the publication in 1887 of Pfeffer's results on the osmotic properties of plant cells and solutions, the osmotic theory of the cell was firmly accepted. Almost any botanist between 1890 and 1936, if asked to state one truism in botany, would probably have said "all mature plant cells are osmometers". Every student during the same period was convinced of the validity of the theory as he or she watched the curling of a dandelion stalk in water. (Even to-day the same experiment is used to demonstrate the theory to first-year students.) Every housewife, not that she realized it, who placed cut stalks of celery in water and saw them curl, was bearing witness to the osmotic theory.

By the turn of the century the theory was to be found in almost all text-books. At that time it was referred to simply as the Osmotic Theory. Later, because of the respectability gained from years of standing, the theory had become the Classical Osmotic Theory. Although it did not explain all the facts known about the water relations of cells, it was generally believed that such facts would eventually fall into place

within the framework of the theory. Moreover, as it is a generalization about the nature of the plant cell, the theory has formed the basis of much fundamental research, not only in water relations, but in many fields of plant physiology. It has provided, for example, the theoretical stimulus for studies on permeability, translocation and cellular organization. However, by the forties of the present century the number of observations concerned with the water relations of cells which were apparently contrary to the "Osmotic Theory" had increased to such an extent that their significance could no longer be overlooked. Many plant physiologists came to doubt the validity of the Osmotic Theory. An alternative theory, the theory of Active Water Uptake, was proposed to explain the apparently anomalous water relations of some plant cells.

This address is an attempt to assess the relative importance and validity of the two theories. Unfortunately, time will not permit a detailed analysis of all the experimental evidence and theoretical arguments which led to the Active Uptake Theory. Only some of the more important so-called "anomalous" data will be examined. This limitation, however, does not lessen the value of the conclusions reached.

At this point it should be mentioned that the Osmotic Theory refers ideally to the mature vacuolated cell. Quite early in the studies of cell water relations two extreme types of cell were recognized (excluding the highly specialized cells such as phloem and xylem): one, the mature, highly vacuolated parenchyma type, characterized by a large central vacuole occupying at least ninety per cent of the total cell volume, enclosed by a very thin layer of cytoplasm and a thin cell wall; the other extreme, the meristematic cell, characterized by dense cytoplasm and the absence of conspicuous vacuoles. Between these types a complete range of cells with varying ratios of cytoplasm:vacuole exist. The pioneer investigators realized that the water relations of the extreme types were not exactly comparable. With meristematic cells it was recognized that the problem was essentially the water relations of cytoplasm, whereas with the mature cell the problem was essentially the water relations of the vacuole. The Osmotic Theory was developed from a study of the water relations of highly vacuolated cells and, strictly speaking, refers in its simple form to the vacuolated cell only. Many of the criticisms of the Osmotic Theory prior to 1936 were concerned with differences between the meristematic and vacuolated cell types. On the other hand, however, most of the recent criticisms of the Osmotic Theory have arisen from studies on mature cells. This fact simplifies our discussion, since the experimental work supporting both the Osmotic and the Active Uptake Theories has been obtained with essentially the same type of cell.

The Osmotic Theory.

According to this theory, a mature plant cell functions like a simple osmometer enclosed by a more or less elastic wall. The vacuolar sap corresponds to the solution phase, the protoplasm or protoplasmic membranes to the semi-permeable membrane, and the cell wall to the more or less elastic wall. The water relations of such a system are defined by the equation

$$DPD = (OPv - OPe) - TP$$

where DPD is the diffusion pressure deficit, OPv and OPe the osmotic pressure of the vacuolar sap and the environment, TP the turgor pressure. Although the movement of water is determined by the direction of the diffusion pressure gradients, the mechanism responsible is osmotic in origin. The protoplasm and the protoplasmic membranes are assumed to play no role, other than that of a passive sieve, allowing the free diffusion of water, but not of solute. It is realized that the above equation is a simplification, in that the distinction between turgor pressure and wall pressure is not made.

The Active Uptake Theory.

The Active Uptake Theory has resulted from the many observations which claim to show that the diffusion pressure deficit is larger than can be expected from the osmotic pressure of the vacuolar sap. This has led to the idea that the diffusion pressure

deficit is due in part to a non-osmotic component as well as an osmotic component. A fraction of the water is assumed to be held in the vacuole by the secretory activity of the protoplasm or the protoplasmic membranes. The equation defining the water relations of the cell has been modified as

$$DPD = (OPv + X - OPe) - TP$$

where DPD, OPv, OPe and TP have the same meaning as before, and X is the non-osmotic or "active uptake component". As the non-osmotic component apparently opposes a diffusion gradient, energy released during respiration is assumed to maintain the state of osmotic non-equilibrium.

As may be seen from this brief comparison, the two theories have one process in common, namely osmosis. They differ in that the Osmotic Theory assumes that the cytoplasm and membranes function only as sieves, whereas the Active Uptake Theory assumes that these structures have a specific function towards water molecules, causing a movement of water independently of osmosis.

The Experimental Basis of Osmotic Theory.

Before discussing the data which have led to the Active Uptake Theory, it is informative to examine some of the experimental results obtained by Pfeffer, de Vries, Fitting, and Overton (see Lucke and McCutcheon, 1932), which form the basis of the Osmotic Theory. As much of this evidence is available in text-books, only the main points will be mentioned. These will suffice to demonstrate the strength of the arguments on which the Osmotic Theory is based. In fact, we shall see there are plenty of examples of cells behaving as almost ideal osmometers.

Pfeffer, whose pioneering research produced the stimulus for the early work on osmosis, became interested in the problem of water relations while experimenting on the mechanism of leaf movement in the sensitive plant. He noticed that certain cells of the pulvinus decreased in volume during movement, at the same time exuding water into the intercellular spaces. After a time lag the cells reabsorbed the water, returning to the original volume, and the leaves returned to their original position. By stretching the contracted tissue, the force necessary to bring about leaf movement was estimated to be from two to four atmospheres. The fact that the cells of the pulvinus underwent large reversible volume changes in which a force of several atmospheres was involved, suggested to Pfeffer that the mechanism might be similar to that occurring in an osmometer. As with the osmometer, the force exerted under tension would be equal to the force causing the movement of water into the cells. Using a Traube type osmometer, Pfeffer determined the quantitative relation between the force causing water to move into the osmometer, that is, the osmotic pressure, and the concentration and the temperature of the solutions.

Following the publication of Pfeffer's results for the osmotic pressure of solutions, van't Hoff formulated his theory of solutions and pointed out the similarities between the Gas Laws and the Laws of Osmosis. This was an important step forward, because it provided the means for testing quantitatively the analogy between the osmometer and the plant cell suggested by Pfeffer. Botanists were now in a position to examine experimentally a definite theory. If the theory were true, certain predictions could be made about the behaviour of cells when placed in solutions. Actually four theoretical propositions were defined.

First, it was argued that if the plant cell functions as an osmometer, then its volume should be the same in all solutions having the same osmotic pressure; or in another form, all solutions isotonic with the cell must have the same concentrations. De Vries used the phenomenon of plasmolysis as a means of estimating the isotonicity of the solutions and the relative osmotic pressure of cells. It was argued that at the point of incipient plasmolysis the concentration of the cell sap must be equivalent to the concentration of the plasmolyticum. The mature vacuolated cells of *Curcuma rubucanales*, *Tradescantia discolor* and *Begonia manicuta* were used as the test osmometers. For non-electrolytes like sucrose and dextrose, the theoretical prediction held, but solutions of electrolytes were found to be isotonic at lower concentrations.

For equivalent concentrations electrolytes were more effective than non-electrolytes. It was clear, however, that towards certain solutions the cells behaved almost as perfect osmotic systems. The discrepancies observed between non-electrolytes and electrolytes were realized by de Vries to be due to differences between the solutions rather than to a property of the cells. To compare the osmotic activity of different solutions, he introduced the term isotonic coefficient i, which is the ratio of the osmotic activity of solutions using as a standard a value of 3 for KNO_3. The fact that solutions have different isotonic coefficients was used as the second argument for testing the hypothesis that plant cells function like osmometers. If the theory were true, then the value of the isotonic coefficient determined plasmolytically should be identical with the value calculated from the freezing point or the conductivity of the solutions. In Table 1 are given some values obtained for *Rhoeo discolor* cells by Fitting.

TABLE 1.

Solution.	Value of *i* Calculated from Plasmolysis.	Cryoscopic.	Conductivity.
KNO_3	1·69	1·78	1·83
KCl	1·74	1·84	1·86
$MgSO_4$	1·05	1·1	1·33
$Mg(NO_3)_2$	2·54	2·55	2·43
$BaCl_2$	2·42	2·46	2·41
$MgCl_2$	2·49	2·64	2·45

The general agreement between the values strongly supports the Osmotic Theory. The discrepancies were interpreted as due to the penetration of solute into the cells. Any plasmolytic method has a disadvantage in that the external solution may have an injurious effect on the living cell, and so cause departures from ideality. But despite the deviations from expectation, the agreement between the values indicates that some cells at any rate obey the van't Hoff Laws.

The third argument used to test the hypothesis was that if cells are osmometers, then it should be possible to use them for estimating the molecular weights of compounds, since isosmotic solutions should contain the same number of molecules. Striking confirmation was obtained by de Vries, who used cells of *Tradescantia discolor* for estimating the molecular weight of raffinose. Several possible values, 396, 594 or 1188, had been proposed. By estimating the concentration of raffinose and sucrose isotonic with these cells, de Vries calculated a value of 595·7 for the molecular weight of raffinose. Subsequently, chemical methods gave the value of 594·3.

Conversely, if plant cells function as osmometers, it should be possible to calculate from the molecular weights the concentrations of solutions isotonic with the cell and with a known sucrose solution. In Table 2 are results obtained by Overton for *Spirogyra* cells.

Again the agreement between the observed and calculated values strongly supports the Osmotic Theory.

The experimental findings of de Vries, Pfeffer, Fitting and Overton, which have been mentioned, clearly demonstrate that some plant cells obey the van't Hoff Law. With certain species the agreement was almost perfect. In others, deviations from ideality were observed. These anomalies were attributed to the penetration of solutes into the cells, to toxic effect of the solutes on the cells, or to errors in the methods. In all instances, whether the agreement was almost perfect or whether larger deviations from ideality occurred, the results appeared to prove that the process controlling the water relations of plasmolysed cells was osmosis, arising from the osmotic pressure of the cell sap. It should be stressed that the results referred to plasmolysed mature

vacuolated cells, that is to cells in which the cell wall can be neglected. The initial evidence on which the simple Osmotic Theory was first based refers to mature, highly vacuolated cells in a particular state, plasmolysis.

Table 2.

Compound.	MW.	Isosmotic Concentration.	
		Observed Percentage.	Calculated Percentage.
Sucrose	342	6·0	—
Mannitol	182	3·5	3·2
Dextrose	180	3·3	3·2
Arabinose	150	2·7	2·6
Erythritol	122	2·3	2·1
Asparagine	132	2·5	2·3
Glycine	75	1·3	1·3

When attempts were made to apply the van't Hoff Law to turgid cells, serious anomalies were observed. Between incipient plasmolysis and full turgor the volume of the cell was smaller than that expected from the osmotic pressure of the cell sap. That the cell wall influenced in some way the volume of the cell had been realized ever since Nageli (1850) described plasmolysis. It was not until much later that a clear picture of the role of the wall in water relations was obtained by Ursprung and Blum, Thoday, Höfler, and others. Their work showed that, as the cell absorbed water, turgor increased due to the opposing force which the cell exerted on the cell contents. The pressure due to the cell wall opposed the osmotic pressure of the cell contents. Hence it was concluded that the property of the cell controlling water relations between incipient plasmolysis and full turgor is not the osmotic pressure, but the difference between the osmotic pressure and the turgor pressure. This quantity has been described by various terms, suction pressure, suction force, water absorbing power, diffusion pressure deficit, net osmotic pressure. In this address, following the suggestions of Meyer (1945), diffusion pressure deficit (DPD) is used.

Since turgor opposed the osmotic pressure, the latter cannot be fully effective in causing water movement and volume changes. As a consequence, deviations from the van't Hoff Law must result. The departures from ideality seen with turgid cells, which at first sight appear to be contrary to the Osmotic Theory, have been accounted for by the turgor pressure. The general relationship between the three variables, the diffusion pressure deficit, the osmotic pressure of the cell sap and the turgor pressure, is given by the equation

$$DPD = (OPv - OPe) - TP$$

where DPD is the diffusion pressure deficit, OPv and OPe the osmotic pressure of the cell sap and the external environment, and TP the turgor pressure.

The equation is applicable irrespective of the exact relationship between turgor and cell volume—a relationship which can be expected to vary according to the elasticity and tensile strength of the cell walls. For some cells the relationship is known to be linear, for others it is more complex. The introduction of a turgor pressure term does not alter the basic concept of the cell as an osmotic system. In fact, the equation is also the equation of an ideal osmometer enclosed by a more or less rigid wall.

Despite the fact that the role of the wall and turgor pressure were recognized by 1920, little effort was made to test the validity of the modified Osmotic Theory experimentally. This oversight can be appreciated because the work of de Vries, Pfeffer and others had shown that many adult cells in the plasmolysed state obey the

van't Hoff Law. Consequently, there were no *a priori* reasons for assuming that processes other than osmosis and turgor would operate in the turgid cells. Most of the experimental work was directed towards establishing the exact relationships between the volume of the cell and the turgor pressure. Such questions as whether turgor is a linear function of volume or not occupied the interests of botanists. The experimental work was not designed to examine the exact nature of the processes contributing to the diffusion pressure deficit. In fact, this appeared to be unnecessary, for it seemed clear that only osmosis and turgor were concerned; that the diffusion pressure term may contain a non-osmotic component was not envisaged.

After 1920 the only important modifications (other than the active uptake hypothesis) to the theory was the realization that the cell is a dynamic, not a static, system—a dynamic system in which both the osmotic pressure and the turgor pressure and the permeability may fluctuate with time and with the metabolic action of the cell. Also the idea of semipermeability was replaced by one of differential permeability. These modifications, however, have not altered the fundamental concept, that the osmotic pressure of the vacuolar sap, and the turgor pressure of the wall are the only factors controlling directly the water relations of a mature cell.

In 1936, following the publication of a paper by Bennet-Clark, Greenwood and Barker, in which the plasmolytic and cryoscopic methods of measuring the osmotic pressure of the vacuolar sap were compared, the subject was reopened. To-day it is one of the controversial issues in plant physiology.

The Evidence for the Active Uptake Theory.

The evidence supporting the "Active Uptake Theory" has come from several different lines of argument. How this evidence appears to contradict the Osmotic Theory may be seen by examining the relationships which might be expected to hold if the Osmotic Theory is correct. We have seen that the Osmotic Theory may be summarized by the equation DPD = (OPv – OPe) – TP. If this is true, then the following relationships are to be expected.

1. When TP = O, i.e. cells in the state of plasmolysis:

(*a*) OPv = OPe, i.e. the osmotic pressure of the vacuolar sap should equal the osmotic pressure of the external plasmolysing solution. This is the theoretical basis of the plasmolytic method of measuring the osmotic pressure of the vacuolar sap. In the plasmolytic method cells are immersed in solutions of varying concentration, and the osmotic pressure of the solution causing incipient plasmolysis is taken as the osmotic value of the vacuolar sap. Furthermore, the value of the osmotic pressure of the vacuolar sap should be the same no matter how it is measured. In particular, the plasmolytic value should be the same as the cryoscopic value. The latter is the value estimated from the freezing point of the cell sap.

(*b*) The volume of the vacuole should be proportional to the reciprocal of the osmotic pressure (1/OPe) of the external solution, and be the same in all non-toxic, non-penetrating solutions.

2. In fully turgid cells, where OPv = TP and DPD = O:

(*a*) Assuming that OPe and TP do not alter, the volume of the vacuole should remain constant with time.

(*b*) The water relations of the vacuole should show the characteristics of a physical, not those of a chemical or metabolic process.

Many observations are known which suggest that for certain tissues these relationships do not apparently hold. In almost every instance the discrepancies between the results observed and those expected suggested that the diffusion pressure deficit was larger than could be expected from the osmotic pressure of the cell sap. For this reason many plant physiologists believe that an energy requiring secretory process in addition to osmosis plays a role in cell water relations.

Some idea of the present controversial nature of the problem of cell water relations can be gauged from the fact that some of the above arguments are identical with those employed by de Vries, Pfeffer and others to establish the Osmotic Theory. In fact, numerous results, of which a few were mentioned earlier in this address, show that many cells obey almost exactly the Boyle van't Hoff Laws. Yet I have just mentioned that more recent work has led to a different conclusion. How can these opposing views be resolved? This question has interested quite a few members of the Botany Department, some of whom are also members of this Society.

In this address, because time is too short, it will not be possible to examine in detail all the evidence which appears contrary to the Osmotic Theory. My analysis of the problem will be limited to a few of the more striking anomalies chosen from the arguments 1 (*a*) and (*b*) to 2 (*a*) and (*b*), outlined previously.

1. (*a*) *The discrepancy between the plasmolytic and cryoscopic values of the vacuolar sap.*

As mentioned previously, the paper by Bennet-Clark, Greenwood and Barker (1936), which triggered the present controversy, dealt with a comparison between the plasmolytic and cryoscopic methods of measuring the osmotic pressure of the vacuolar sap. According to the arguments already outlined, the plasmolytic and cryoscopic values should be identical, if the Osmotic Theory is correct. For several different plants the plasmolytic value was found to be from 2·2 to 7·1 atmospheres greater than the cryoscopic value. To account for this discrepancy, Bennet-Clark *et al.* suggested that the cryoscopic value, since it was determined on sap expressed under pressure from the tissues, measured the osmotic pressure, OPv, of the vacuolar sap, while the plasmolytic value, since it was determined on living cells, measured the total water-absorbing power of the tissue. The latter quantity would include all processes concerned with the uptake of water. They attributed the difference between the two values to a non-osmotic secretory component, X, so that the plasmolytic value was assumed to be a measure of OPv + X.

Subsquently, other investigators confirmed the observations of Bennet-Clark *et al.* on different tissues. Although the existence of a discrepancy between the two values has been confirmed many times, there is no general agreement on the explanation. Nor is the plasmolytic value invariably greater than the cryoscopic.

There is no *a priori* reason for assuming that the discrepancies must be due to the same cause in the different experiments. It is possible that the explanations suggested by the various authors may be the true explanation for the particular experiment described. Actually the crux of the problem depends on the validity of the assumptions that both methods have a high degree of accuracy, and that the expressed sap used for the cryoscopic measurements is pure vacuolar sap. There is a mass of evidence demonstrating that both methods are beset with errors, and that the expressed sap used in cryoscopy is not pure vacuolar sap (Crafts, Currier and Stocking, 1949).

Let us look at some of these errors. In the techniques of sap extraction, either a small mass of living material or a mass of tissue previously killed in liquid air or some other agent is wrapped in cheesecloth and pressed. The liquid, which is expressed under high, rapidly applied pressures, is taken to be pure vacuolar sap.

Mason and Phillis (1939) and Bennet-Clark *et al.* (1936) claim that high, rapidly applied pressures rupture the cell membranes or cause fissures to develop through which pure vacuolar sap escapes. The main argument used to support this view is the relationship observed between the concentration of the expressed sap and the applied pressures. Small, slowly applied pressures yield a dilute sap, whereas a high, rapidly applied pressure suddenly yields a concentrated sap. Low pressures, so it is argued, do not destroy the permeability properties of the tonoplast and cell membrane; consequently filtration of the expressed sap occurs. The low cryoscopic values always observed with low pressures are consistent with this argument. High pressures, suddenly applied, are assumed to destroy the permeability properties of the membrane, and so allow pure sap to escape. Hence the high cryoscopic value which is suddenly obtained with high pressures. This assumption overlooks the possibility of the cytoplasm being

disorganized by high pressures. If disorganization occurs, the possibility of contamination of the vacuolar sap by cytoplasmic materials must exist. Since there are no sound reasons for neglecting this possibility, it becomes of some importance to speculate on the possible effects of high pressures on the distribution of water between the components of the disorganized cell. Presumably, in a living tissue at equilibrium with its environment, the activity of the water in the vacuole, cytoplasm, and cell walls must be equal. If the Osmotic Theory is correct, the activity of the water in the vacuole is determined by the concentration of the solutes present, whereas, in the cytoplasm and wall, because of the presence of colloidal materials, the activity of the water would be due to imbibitional forces as well as to any freely diffusible solutes present. Van der Waal forces, hydrogen bonds, dipoles and ions held electrostatically to charges attached to colloidal components are taken collectively as imbibitional forces. Further, the activity of the water in all phases of the cell is influenced by the hydrostatic pressure of the cell contents.

In such a system, when pressure is applied, movement of water will not occur until the applied pressure equals the hydrostatic pressure. Thereafter, provided the pressure does not disorganize the cell membranes or the cytoplasm, the activity of the water in each phase of the cell will be insufficient to maintain the initial water content, and pure water must exude from the tissue. The fact that low pressures are known to yield almost pure water supports this contention.

With high pressures the situation is likely to be different. Since the plasmolytic-cryoscopic discrepancies have been observed with sap extracted from both living and tissue killed in liquid air, etc., before the application of pressure, it seems reasonable to assume that filtration of solutes from the vacuolar sap by the cell membranes is not a main factor contributing to the difference. Also, certainly with killed tissue and most probably with the living disorganized tissue, it is difficult to escape the conclusion that sap extracted under high pressures must be a mixture of cytoplasmic, vacuolar and wall fluids. We have seen that before the application of high pressures and before killing the water in the vacuole is associated with diffusible solutes and is in equilibrium with the water in the wall and cytoplasm which is held partly by imbibitional forces. Hence in the killed or disorganized cells mass some of the water will be associated with "free" solutes and the rest will be held by imbibitional forces but the relative distribution may not be the same. Is it reasonable to assume that the concentration and composition of sap expressed under pressure will remain identical with that of the vacuolar sap of the living tissue?

Actually, many indirect arguments can be stated to the contrary. For instance, in vacuolar sap containing organic acids or other solutes capable of undergoing dissociation, changes in the hydrogen ion concentration would alter the degree of dissociation, and so alter the freezing point of the sap. That changes in the pH of the vacuolar sap may occur cannot be rejected, since there is evidence that the vacuolar solution and the cytoplasm in the living state are at different pH's. Also, exposure to atmospheric oxygen and carbon dioxide and autolysis changes can be expected to alter the pH of the mixed fluids. Changes caused in this way could either increase or decrease the freezing point of the expressed sap, depending on the direction of the pH change.

Another factor which could alter the freezing point of the expressed sap would be changes in the electrostatic properties of the cytoplasmic and wall colloids and therefore changes in their imbibitional properties. If death or disorganization does not alter the total number of electrostatic charges, hydrogen bonds and so on, the fraction of water held by these imbibitional forces in the living tissue would be expressed under pressure as almost pure water. The applied pressure would oppose the imbibitional pressure, allowing the water but not solutes to escape. This would result in a dilution of the vacuolar sap with a decrease in the freezing point of the expressed sap. On the other hand, if disorganization increased the electrostatic properties of the colloidal components, water would be absorbed and the freezing point of the vacuolar sap increased.

An increase in the freezing point of the expressed sap could also result from the adsorption of vacuolar solutes onto the colloidal components of the cytoplasm and cell wall.

Information on the water relations of the cytoplasm is badly needed. What is required is accurate information on the ratio of water held by diffusible solutes and that held by fixed charges. Also information is needed about the role of metabolism in the maintenance of the fixed charges which are the basis of the imbibitional properties of the cytoplasm. If, for instance, the electrostatic properties are maintained in some way by metabolism, then the activity of water in living and dead cytoplasm can be expected to be different—probably less in the latter.

All the factors briefly mentioned cannot be neglected when considering the nature of the expressed sap. In fact, the chance of the expressed sap resembling the vacuolar sap of living tissue seems remote. Unfortunately, few experiments aimed at assessing vacuolar contamination have been made. It is to be expected that contamination should vary according to the ratio of cytoplasm:vacuole. The data existing are conflicting. With young cells, all cytoplasm, the discrepancy between the plasmolytic and cryoscopic values is small (Currier, 1944), whereas with leaf tissue, having a high cytoplasm:vacuole ratio, the discrepancies are very large (Mason and Phillis, 1939). With the large coenocytic *Nitella* cells, where the expressed sap is most likely to be pure vacuolar sap, the discrepancy is very small (Wildervanck, 1932).

Just how much contamination of the vacuolar sap by vacuolar and wall materials occurs during extraction is, at the present moment, impossible to assess; but that contamination must occur cannot be denied. Mostly the errors we have considered would give an underestimate of the real cryoscopic value for the vacuolar sap.

On the other hand, the errors which have been claimed to exist in the plasmolytic method lead to an overestimate of the plasmolytic value. First, detection of the point of incipient plasmolysis is not easy. In fact it may not be observable immediately the turgor pressure becomes equal to zero, and a slightly higher concentration may be needed to make plasmolysis visible. Also, according to Buhmann (1935) yet higher concentrations, as much as x atmospheres, must be used to overcome the adhesion of the cytoplasm to the cell walls. Levitt, however, concluded that adhesion forces are negligible, since he could detect no difference between the plasmolysis value obtained by plasmolysis and by deplasmolysis.

Another possible source of error is concerned with the volume-correction factor used in comparing the plasmolytic and cryoscopic values. Usually the two values are determined on cells at different degrees of turgor, the cryoscopic on turgid cells and the plasmolytic on cells at incipient plasmolysis. The two values have to be corrected for the difference in the volume of the tissue at the different states of turgor. Most investigators have used a correction factor of *ca.* five per cent. Mainly owing to the difficulty of measuring cell volumes, very few accurate measurements of cell volumes at different degrees of turgor have been made. Recently, Mercer (1950), using a newly developed technique of measuring the osmotic volume of tissues, found differences as high as twenty per cent. between the volumes at full turgor and incipient plasmolysis. Consequently, it is possible that the correction factor used in the plasmolytic-cryoscopic comparison is much too small.

A pointer in this direction is seen in the results of Currier (1944), who showed that the discrepancy between the two values decreased from 2·7 atmospheres for sap extracted from turgid cells to 1·1 atmospheres for sap extracted from cells at incipient plasmolysis.

A puzzling feature of the discrepancy between the two values is the tremendous variation observed between different species and different samples of the same species. No satisfactory explanation of such variability has yet been offered. Depending on the point of view one wishes to take, the variability could reflect variations in the magnitude of errors in the methods or in the magnitude of an active water uptake

process. One final point worth mentioning is that the discrepancy is usually greatest in old, senescent tissues, and smallest in actively growing tissues. Yet it could be argued that an active uptake process should be greatest in young tissue where growth is active.

From what has been said of the possible errors in the two methods, conclusions drawn from the discrepancy between the plasmolytic and cryoscopic values should be accepted with caution. In fact until the plasmolytic method can be compared with cryoscopic measurement using sap which is known to be pure vacuolar sap, the conclusions must be accepted with reservations. Until this is done, the discrepancies between the two methods should not be used as evidence of an active water uptake mechanism, and hence as a basis of criticism of the Osmotic Theory.

1. (*b*) *Anomalous volume:pressure relationships.*

The second piece of evidence which has been used to support the "Active Uptake Hypothesis" concerns the anomalous volume behaviour of plasmolysed cells when transferred from one isosmotic solution, sucrose, to another, KCl. In this type of experiment epidermal cells of the onion bulb scale or similar tissue are plasmolysed first in sucrose solution and, after equilibrium is attained, transferred to an isosmotic solution of a salt, say KCl. The volume of the protoplast is recorded throughout. We have pointed out earlier (relationship 1 (*b*)) that according to the osmotic theory the volume of the vacuole should be the same in all isosmotic non-penetrating solutions. In the transfer experiments being considered several investigators (Bennet-Clark; Mercer) have shown that the volume does not remain constant. The volume of the protoplast undergoes spectacular expansions and contractions which would appear to be inexplicable on the osmotic theory. Bennet-Clark and Bexon (1940) realized that such volume changes might occur if the cell walls were differentially permeable to the solutes used. If sucrose diffused slowly and KCl rapidly through the cell walls, temporary changes would occur in the solution between the protoplast and the cell walls. As a result volume changes would occur. Gradually, as the solutes reached equilibrium, the volume would return to the initial volume. This possibility was rejected following the discovery that isolated protoplasts, no cell wall present, underwent apparently similar volume changes. This result, plus some theoretical reasons concerning the diffusivity of substances in cell wall, led Bennet-Clark and Bexon to conclude that the anomalous behaviour must be due to some process of the protoplast. They assumed the process to be the "active water uptake" process. Moreover, because the phenomenon was associated with solutions of electrolytes and non-electrolytes, they postulated that the active process was analogous to electrosmosis. The anomalous behaviour was explained as due to the electrolyte and non-electrolyte solutions altering the potential difference across the cell membrane, and thereby altering the rate of water movement due to electrosmosis.

Later work has shown that the apparently anomalous behaviour of the protoplasts enclosed in the epidermal cells can be explained on the basis of the differential permeability of the cell wall without involving electrosmosis or an active process. As mentioned, the wall explanation was rejected by Bennet-Clark *et al.*, because isolated protoplasts underwent apparently similar volume changes when transferred from sucrose to salt and salt to sucrose. Actually the behaviour of isolated protoplasts as shown by Mercer (1950) is not analogous to the behaviour of the tissue protoplasts. In Mercer's experiments the behaviour of the isolated protoplasts was shown to be due to the protoplasts undergoing changes in shape, not volume. Shape changes can be interpreted as volume changes since under the microscope the apparent diameter is measured and the volume calculated on the assumption that the protoplasts remain spherical throughout the experiment. The apparently large expansions in volume associated with the transfer from salt to sucrose reflected the fact that the protoplast changed from a sphere in KCl to a flattened disc in sucrose. Moreover, it was shown that the changes in shape were caused by the difference in density between the protoplast and the solutions and by the tendency of the protoplast to be distorted at the sucrose-air interface. When the protoplasts were prevented from contacting the sucrose-air interface the large apparent volume expansions did not occur.

The importance of the cell wall in causing the volume changes of the protoplasts within the cell walls was demonstrated by perforating the cell walls at the ends of the plasmolysed protoplast. During transfer the volume changes of the protoplast in the treated cells were almost negligible. These results suggest that the volume changes induced in onion epidermal protoplasts by rapid transfer from one isosmotic solution to another can be explained in terms of the osmotic theory. The fact that the same tissue was used by Bennet-Clark and Bexon strongly suggests that their results may have a similar explanation.

At this point Levitt's (1936) observations on the volume:pressure relationships of the mature vacuolated protoplast of the parenchyma cells of the onion bulb scale became relevant. Levitt found that the volume of the protoplasts was inversely proportional to the osmotic pressure of the external medium. In other words, the mature protoplasts behaved like simple osmometers. Similar results were obtained by Mercer (1950) and Clark and Mercer (1955), using protoplasts isolated from the epidermal cells of the onion bulb scales. In addition, Mercer (1950) and Clark and Mercer (1955) showed that the volume of the protoplast was independent of temperature. A result which is consistent with the view that the volume of the protoplasts is maintained by the osmotic pressure of the cell sap and the osmotic pressure of the external solution. These protoplasts were isolated from the same tissue-type, epidermis, and the same species of plant as that used by Bennet-Clark and Bexon and Mercer to demonstrate the anomalous volume:pressure changes during salt:sugar transfers.

Similar volume:pressure:temperature relationships were observed by Mercer and Clark and Mercer, using the mature vacuolated protoplasts isolated from the petiole of *Begonia* sp., the epidermis of *Tradescantia* sp., the parenchyma cells of Beetroot and Carrot root tissue, and the parenchyma of the peduncle of Iris flowers. These results, for a number of protoplasts isolated from different species, demonstrate the importance of osmosis in the water relations of at least these protoplasts. In fact, these protoplasts functioned like simple osmometers and obeyed, with considerable accuracy, the Boyle van't Hoff Law.

Thus there seems little argument for accepting as evidence for the "Active Uptake Hypothesis" the so-called anomalous volume changes induced in onion epidermal protoplasts (also observed in other tissue) by rapid salt:sugar transfer. In actual fact the behaviour of these cells really provides a striking confirmation of the Osmotic Theory.

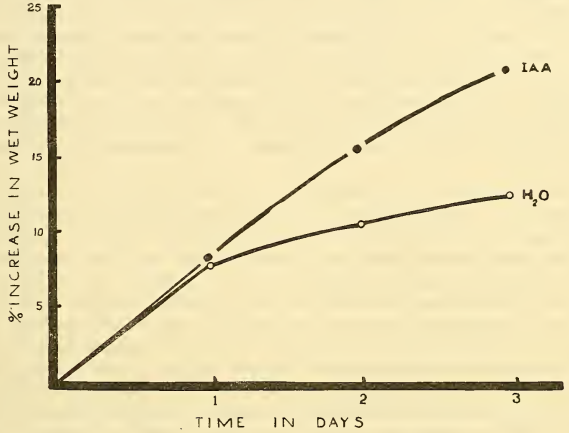
2. (*a* and *b*) *The anomalous water uptake of tissues in which the diffusion pressure deficit is zero.*

Finally, there is the considerable body of evidence supporting the active uptake theory obtained with tissues in which the diffusion pressure deficit is "zero". This evidence is contrary to the relationships 2 (*a*) and (*b*) described earlier. In these experiments pieces of homogeneous tissue such as the parenchyma tissue of tubers and tuberous roots are removed from the organs and stored in aerated water or solutions for several days. From time to time the wet weight is determined and used as a measure of the water content of the protoplasts. By adding various substances to the external solutions the effect of metabolites, auxins, salts and temperature on the water relations of the tissue has also been studied.

If the Osmotic Theory is correct, tissue immersed in a solution should be in equilibrium with the external solution when the diffusion pressure deficit is zero or when it is equal to the osmotic pressure of the external solution. Under these conditions one might expect the volume of the tissue to be independent of time. This expectation does not hold. In general the volume as measured by the wet weight increases with time. This is illustrated by data for the Jerusalem artichoke tuber tissue obtained by McLaren and Mercer (1955) (Text-fig. 1).

All investigators agree that the rapid increase in wet weight observed during the first four to eight hours after immersion in a solution is due to the penetration of water or solution into the intercellular spaces and to the uptake of water by the cells

following the release of tissue tension. After this initial adjustment is complete the diffusion pressure deficit should be zero and no further uptake of water is to be expected. Yet over a period of several days the wet weight increases. Whether this behaviour is inconsistent with the osmotic theory is the point at issue. If either the osmotic pressure or the turgor pressure or both change with time, the diffusion pressure deficit would not remain zero, and the water uptake arising in this way would be consistent with the osmotic theory. Hence before the osmotic theory can be rejected it must be proved beyond doubt that either or both the osmotic pressure and turgor pressure do not alter with time.



Text-fig. 1.—The percentage increase with time in wet weight in grams of Jerusalem Artichoke tissue stored in 10^{-5} molar indol acetic acid I.A.A. and water.

A few typical examples of the experiments and reasoning claimed to disprove such possibilities will be given.

Reindeers (1938) reported the stimulatory effect of auxin on the increase in wet weight and decrease in dry weight of tissues stored in water. Changes in dry weight were used as a measure of the respiratory activity of the tissue. It was argued that, because the increase in wet weight was dependent upon aerobic conditions and because this increase was stimulated by auxin, which also stimulated the loss of dry weight, the increase in wet weight must be controlled by a metabolic process maintained by energy released by respiration and not by osmosis.

Similar conclusions have been reached by other investigators (see Crafts, Currier and Stocking, 1949). Steward, Stout and Preston (1940) criticize Reindeers' assumption that changes in dry weight measure respiration and are of the opinion that her data do not necessarily exclude osmosis. At the same time, however, they conclude from their own data that some kind of active water uptake process occurs in potato tubers kept in aerated KBr and KNO_3 solutions in the absence of auxins. Tissue kept in the salt solutions had a greater fresh weight than those in distilled water. The addition of Ca^{++} ions caused a decrease in the wet weight. The increase in wet weight was correlated with respiration and protein synthesis. These results led them to suggest "that actively metabolizing cells which can grow may absorb water in a manner which has but little relation to any conventional osmotic or suction pressure theory, but may be more directly linked with metabolic processes (respiration and protein synthesis)—processes which are determined by oxygen and affected by the nature of the salts present in the external solution".

The essential point supporting the Active Uptake Hypothesis obtained with the above type of experiment is the fact that the increase in wet weight has the physiological characteristics of a metabolic process, not those of a physical process such as osmosis. It should be remembered, however, that the physiological characteristics may reflect metabolic processes concerned with the maintenance of the osmotic properties of the tissue.

More direct efforts to determine the contribution of osmotic pressure changes in the vacuolar sap have been made by following the cryoscopic value of the sap as the wet weight increases. In general the results indicate that the cryoscopic value tends to remain constant or to fall slightly. The data obtained by van Overbeek (1944) will serve to illustrate the point. Auxin-treated tissues had a lower osmotic pressure than the controls in water. As the difference between the osmotic pressure of the treated and control tissues could be accounted for by the extra water in the auxin-treated tissue, van Overbeek concluded that there was no change in the total solute content of the tissue. Hence it seemed improbable that changes in the osmotic pressure of the cell sap could have been responsible for the increase in the wet weight of the tissues. It was concluded that either changes in turgor or a non-osmotic water uptake process was concerned.

Another approach to the question of the roles of turgor and osmotic pressure changes, has been the study of the water relations of tissues in hypotonic solutions. Many investigators have shown that the wet weight of tissue in hypotonic solutions may increase even though plasmolysis has occurred. This fact is shown by results obtained by Commoner, Fogel and Muller (1943), who found that auxin could prevent the loss of weight by tissue immersed in 0·2M. (hypotonic) sucrose solutions. Addition of potassium chloride and fumarate increased the wet weight further. The authors concluded that the water uptake was associated with salt accumulation, and that auxin and the organic acids stimulated water uptake indirectly via the salt accumulation process. Such an explanation overlooked the fact that wet weight increases occur in distilled water and auxin solutions—no salt present. Thus, although salt accumulation may be a contributory process under some circumstances, it cannot be the general explanation.

The importance of the experiments using hypotonic solutions lies in the two conclusions which have been drawn from the results. First, since the cells were plasmolysed turgor changes can be rejected as a cause of the increases in wet weight. Secondly, the increase in wet weight must have occurred against an osmotic pressure gradient. These conclusions would appear to eliminate the possibility of osmotic factors playing a role in the increase in wet weight of tissues in hypotonic solutions. Consequently the increase in wet weight has been attributed to an active uptake process.

More recently, Bonner, Bandurski and Millerd (1953) examined the effect of auxin on the wet weight of tuber tissue of Jerusalem artichoke in both hypertonic and hypotonic solutions.

They confirmed the earlier observations that wet weight increases can occur in hypertonic solutions, that is, where turgor effects do not operate and where the increase in wet weight must have occurred apparently against an osmotic gradient. In addition, they showed that auxin stimulated respiration. Clearly, since the tissue was plasmolysed the auxin effect could not be via changes in the cell wall. Bonner *et al.* concluded that the auxin acts via the active uptake mechanism, which is linked to respiration.

During the last ten years or so it has been shown conclusively that energy-requiring cell processes are linked to respiration via the phosphate bond transfer mechanism. The substance dinitrophenol is known to uncouple the link between respiration and the energy transfer mechanism. Hence by treating tissue with dinitrophenol processes requiring energy are inhibited. As an example of this, one can mention Robertson's (1951) work showing the inbibition of salt uptake by 10^{-3}M. dinitrophenol. Bonner *et al.* argued that if water uptake is an active process, then treatment of tissue with dinitrophenol should inhibit the process. Experiments were in agreement with the

hypothesis. Treatment of tissue with dinitrophenol abolished the increase in the wet weight in both auxin-treated and control tissue. They concluded, therefore, that the increase in wet weight of the tissue must have been caused by an active uptake process.

Further support for this idea of an active uptake process playing a role in water relations has come from experiments using coleoptile segments in place of discs of tissue. It is sufficient to mention one or two experiments. Kelly (1947) showed that auxin increased both respiration and the wet weight of coleoptile segments under aerobic but not anaerobic conditions. This behaviour is identical with that observed for storage tissue. In addition, it was argued that if an active uptake process maintained by respiration exists, then inhibition of respiration should inhibit water uptake. It was found that the respiratory inhibitors azide and iodo-acetate prevented the increase in wet weight. Hence it seemed reasonable to assume that an active uptake process is concerned with the increase in wet weight of coleoptile segments.

Similar conclusions have been reached from experiments on the nature of root presssure (Rosene, 1944; van Overbeek, 1942). Briefly it has been shown that the rate of exudation from decapitated roots is depressed by respiratory inhibitors such as KCN, is temperature dependent, and depressed by anaerobic conditions. Also van Overbeek made the interesting observation that the osmotic pressure of mannitol solutions required to stop exudation from tomato roots was always from fifty to seventy per cent. greater than the osmotic pressure of the exudate. The difference was interpreted as due to an active uptake process. On treatment of the roots with 10^{-4}M. KCN the discrepancy was reversibly inhibited. Such behaviour strongly indicates that a metabolic process is concerned. It is but a short step to assume that the metabolic process is an active water uptake process dependent upon respiration.

At this point it is useful to summarize the evidence supporting the Active Uptake Hypothesis. Certain experimental results suggest that the increase in the wet weight of tissues stored in water or certain solutions cannot be attributed either to changes in turgor or changes in the osmotic pressure of the vacuolar contents. On the other hand, the increase in wet weight has the following physiological characteristics: (1) a high temperature coefficient, (2) dependent upon aerobic conditions, i.e. O_2, (3) stimulated by auxin, which also increases respiration, (4) is inhibited by respiratory inhibitors such as azide, iodacetate, potassium cyanide, and is (5) inhibited by dinitrophenol, which uncouples the energy transfer mechanism. These observations would appear to provide strong evidence for a metabolic process. Consequently many plant physiologists accept the view that an active non-osmotic component plays a role in the water relations of such widely different processes as root pressure, coleoptile segments and parenchyma tissue slices.

In any controversial issue there is always the opposite view as well as conflicting experimental results. Levitt (1947), for example, using potato tissue and the same technique as used in the other experiments with tissue slices, found that the respiratory inhibitor (KCN) did not inhibit the auxin-induced increase in wet weight of the slices. Also, contrary to Stile's result, he found that the wet weight was not dependent on the temperature. Reduction of the temperature from *c.* 25°C. to 1°C. did not cause a loss of water from tissue previously stored at 25°C. Conflicting results are known also for root pressure. Skoog, Broyer and Grossenbach (1938) found no correlation between respiration and the rate of exudation from decapitated sunflower roots. Burström (1953) has criticized the results obtained by Bonner, Bandurski and Millerd, claiming that the permeation of mannitol increased the osmotic pressure of the vacuolar sap and as a result water was absorbed along an osmotic gradient. Taken alone, such conflicting results cannot be regarded as sufficiently strong evidence for rejecting the active uptake hypothesis as applied to wet weight data. Time does not permit a complete analysis of all the data obtained in wet weight experiments. Consequently only a critical assessment of the more significant experiments will be attempted.

Burström, in repeating the experiments of Bonner *et al.*, measured not only the wet weights but also the cryoscopic values of the tissues. The final cryoscopic values were found to have increased almost proportionally with, and to be greater than, the

osmotic value of the external solutions. Since the final values were greater than the osmotic values of the solutions, Burström concluded that the increase in wet weight must have occurred in the direction of an osmotic gradient, not against a gradient as claimed by Bonner *et al.*, and others. Moreover, he argued that the extra osmotic pressure of the vacuolar sap must have been due to the penetration of mannitol into the vacuoles. The possibility that it was due to solution held in the intercellular spaces was rejected since the final cryoscopic values were always greater than the initial value plus the osmotic value of the particular solution. Since Bonner *et al.* used the same type of tissue and solutions Burström concluded that their results must have been caused also by the penetration of mannitol, and therefore have little value as evidence for the active uptake hypothesis.

Burström's conclusions are extremely important, for, if correct, most of the evidence supporting the "Active Uptake Theory" based on the water relations of tissues in hypotonic solutions must be rejected. In view of this it becomes essential to consider his results in more detail.

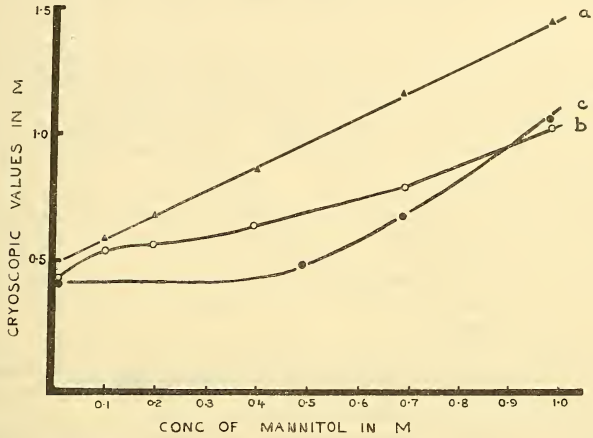
The point made by Burström from his results is that the cells became permeable to mannitol, which increased the osmotic pressure of the cell sap, leading to an osmotic absorption of water. Before examining this conclusion it is necessary to consider the processes which are known to occur when a cell is placed in a solution of a permeable substance. Initially there is a loss or gain of water depending upon the concentration of the solution and the initial diffusion pressure deficit of the cell. Plasmolysis may or may not occur. Then, as penetration commences, the osmotic pressure of the cell sap gradually increases, leading to the absorption of water. These processes continue until equilibrium between the cell and the external solution is attained. Equilibrium occurs when the internal and external concentrations of the permeable substance are equal, and when the diffusion pressure deficit is zero. The diffusion pressure deficit becomes zero at full turgor.

An examination of Burström's results, the wet weight time curves, shows that equilibrium had almost been attained after one hour. Consequently it seems reasonable to assume that equilibrium must have been established after fifty-four hours. Hence if permeation occurred, as claimed, then the concentration of mannitol within the cells should have been equal to the concentration of mannitol in each solution. In addition, the cells should have been fully turgid in all solutions. Under these circumstances one would have expected the wet weights of the fully turgid cells to have been the same in all solutions. Actually the results show that the wet weights decreased as the concentration of the external solutions increased. This observation really proves that permeation could not have occurred.

However, Burström argued that permeation must have occurred because the cryoscopic value of the tissue increased more or less proportionally with the osmotic pressure of the external solutions. We have seen that if permeation occurs, then, at equilibrium, the internal and external concentrations of the penetrating substance must be equal. Since in Burström's experiments equilibrium had been attained the cryoscopic values of the cell saps should have been equal to the initial cryoscopic value, 0·495M., corresponding to the cryoscopic value of the cell sap plus the cryoscopic value for the particular solution. For example, the cryoscopic value of the tissue immersed in the 1·0M. solution should be 1·495M. and that of the tissue in 0·5M., 0·995M. Curve *a* (Text-fig. 2) shows the cryoscopic values expected for all solutions. Actually the values observed were much less (curve *b*. Text-fig. 2). The difference between the two curves argues against permeation.

It is interesting to estimate the cryoscopic values which one might expect assuming no penetration. Under these conditions, for tissues at equilibrium, the diffusion pressure deficit of the tissue must be equal to the osmotic pressure of the external solution in each solution. Since the initial cryoscopic value of the tissue was at 0·495M., one can assume that plasmolysis would have occurred in the 0·5M. solutions. In the more concentrated solutions 0·7M. and 1·0M. exosmosis of water would have increased the diffusion pressure deficit to 0·7M. and 1·0M. respectively. In the more dilute solution

a gain or loss of water, depending on the value of the initial diffusion pressure deficit of the tissue, is to be expected. From the data the initial diffusion pressure deficit would appear to have been at *c.* 0·2M., since no gain or loss of weight was recorded in this solution. Unfortunately, not knowing the magnitude of the actual changes in volume of the tissue, gain or loss, occurring in the solutions 0·495M. to water, it is not possible to predict accurately the cryoscopic value expected for the tissues in these solutions. All that can be done is to indicate that the value in water would be a few per cent. less than 0·495M. through dilution, and approaching 0·495M. in the



Text-fig. 2.—The cryoscopic value of the tissue plotted against the concentration of mannitol in M. Curve *a*: Values expected if complete permeation of mannitol. Curve *b*: Values observed. Curve *c*: Values if no permeation of mannitol.

more concentrated solutions. The difference in wet weight between the tissue in water and the 0·2M. solution was only *c.* two–three per cent. The cryoscopic values expected if no permeation occurred are shown by curve *c* (Text-fig. 2). Burström expressed surprise that the cryoscopic values of the expressed sap increased proportionally with the external concentration of the solutions, and concluded permeation must have occurred. As shown by curve *c* (Text-fig. 2), a similar relationship also arises without permeation. These values, however, are less than those obtained by Burström.

The theoretical limits for the cryoscopic values expected for the expressed saps for either complete permeation or for no permeation at all are both different from the observed results (curves *a*, *b*, *c*, Text-fig. 2). The observed result falls approximately midway between the two. It must be remembered that we are dealing with tissue at equilibrium, therefore one of the above results should have been obtained. In view of the fact that the wet weights of the tissues decreased, that is, the tissues were not at full turgor, curve *c* for no permeation is taken to be the correct theoretical limit for the cryoscopic values. The explanation of the differences between the curves is not immediately apparent. It is not improbable that it lies in the assumption that sap expressed under pressure is pure vacuolar sap.

A note of warning concerning the use of molar and molal solutions can be inserted at this stage. It is by no means clear, from the results in the majority of papers published on water relations of tissues in hypotonic solutions, which type of solutions were used. For example, apparently the symbol M. used by Bonner *et al.* referred to molar solutions, since the same symbol was used for the auxin solutions as well as the hypertonic solutions. On the other hand Burström, certainly as far as the cryoscopic

values of the expressed saps are concerned, used the symbol M. for molal solutions. One cannot decide from his results whether the M. used for the mannitol solutions refers to molar or molal. To avoid confusion and error, investigators should indicate the meaning of the symbol M. used when expressing concentrations. For example, a molar solution of mannitol is equivalent to a *ca.* 1·13 molal solution. Comparison of results obtained using the different types of solutions could lead to apparent discrepancies.

Although no explanation can be offered for the larger than expected cryoscopic values obtained by Burström, the main fact remains that the results do not support the conclusions. To the contrary, they indicate that permeation could not have occurred. Hence one must reject Burström's evidence as a basis of criticism of the "Active Uptake Theory".

Yet the observation that the wet weight does not decrease to the extent expected on osmotic grounds remains. In Bonner's experiments incipient plasmolysis was at 0·12M., hence one might expect the wet weight to have decreased by half in a 0·24M. solution. Similarly in Burström's experiments the wet weight should have decreased by half between 0·5M. and 1·0M. This anomaly is the essential evidence on which the "Active Uptake Theory" rests.

In all the research on the water relations of tissue slices in hypotonic solutions which has so far been published no measurements of cell volume have been attempted. Invariably it has been assumed that wet weight can be used as a measure of the volume and water content of the protoplasts of the tissue. The validity of this assumption has never been questioned. The error arising from this oversight is well shown by some of the results obtained by persons working in the Department of Botany at the University of Sydney.

McLaren and Mercer (1955), using a technique based on Archimedes' principle—a body immersed in a solution displaces its own volume of solution—have compared the apparent osmotic volume of tissues immersed in solutions of different concentrations with the wet weights of the same tissues.

The Apparent Osmotic Volume is essentially a measure of the total volume of the protoplasts in a tissue. The technique was developed by Professor G. E. Briggs, of the Botany School, University of Cambridge, England, and is reported fully in a dissertation for the Ph.D. degree submitted to that University by the present author (1950).

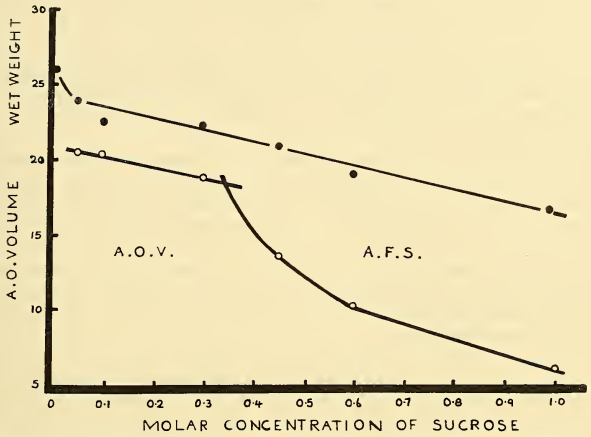
From Text-figure 3 it can be seen that, except for dilute solutions, wet weight is not a measure of volume. Essentially similar results have been obtained for potato, carrot, beetroot and Jerusalem artichoke tissue. The reason for the lack of correlation is obvious. After plasmolysis the Apparent Free Space of the tissue increases as the protoplasts shrink. Volume previously occupied by the protoplasts becomes filled with the external solution. The wet weight, therefore, includes not only the weight of the protoplasts, but also the weight of the solution in the Apparent Free Space. It is easy to see how wet weight measurements could give the impression that water uptake can occur from hypotonic solutions. We feel that many of the results so far published for the water relations of tissues in hypotonic solutions can be explained in this way. One must conclude that most of the data published by van Overbeek, Bonner *et al.*, Burström, and others have little meaning in so far as the problem of water relations is concerned.

Much of the variability in the magnitude of the so-called uptake obtained by different investigators may also be readily explained as due to variations in the rigidity of the cell walls and to the pressure used for drying the tissues. It is to be expected that tissues with rigid walls, which would contract less following plasmolysis and which would resist compression during drying, would show an apparently large uptake. The importance of the pressure used when drying tissues for wet weight measurements has been clearly demonstrated by Ashby and Wolf (1947).

Another extremely important finding arising from the Apparent Osmotic Volume experiments is the form of the volume:pressure relationships. One of the relationships expected from the Osmotic Theory is that the volume of a plasmolysed cell should vary inversely as the reciprocal of the osmotic pressure of the external solutions. The

Apparent Osmotic Volume, after plasmolysis, does in fact obey such a relationship. Thus when volumes, not wet weights, are considered, the water relations of tissues in hypotonic solutions support rather than disprove the Osmotic Theory.

Although the lack of correlation between wet weight and volume invalidates most of the conclusions previously reached from tissue slices in hypotonic solutions, it is most unlikely to be the explanation of the increase in wet weight observed for tissues stored in water, auxin solutions or very dilute solutions. In these solutions the tissues would be turgid, and the wet weight, apart from the small amount of solutions in the



Text-fig. 3.—The relationship between the wet weight in grams and concentration of the external solution ·—· and the relationship between the apparent osmotic volume in c.c. and concentration of the external solution o—o.

intercellular spaces which presumably would not alter with time, should give a reasonably accurate measure of cell volumes. The explanation of the increase in wet weight in water, and the stimulatory effect of auxin must be sought elsewhere. It must be emphasized, however, that before rejecting the Osmotic Theory, rigid proof that changes in either or both the osmotic pressure of the cell sap and the turgor pressure do not occur. The fact that with the tissue slice technique most experiments last for several days at least, the possibility of changes in these quantities must not be overlooked.

These possibilities have long been realized, but the experimental evidence of their significance is conflicting. In general, the results show that the osmotic value of the sap tends to remain more or less constant, or to decrease slightly as the wet weight increases. Despite the conflicting nature of the results, and remembering that osmotic values are frequently determined by the cryoscopic technique, there is a strong indication that the osmotic pressure tends to remain constant. This is an extremely important possibility, for, if proven, it means that the total number of osmotically active particles must increase along with water uptake. Or in other words, water uptake follows the synthesis of osmotic material.

With turgid cells the volume and, therefore, the area of the cell walls increase during water uptake. This implies a stretching of the walls. Consequently, changes in wall elasticity cannot be overlooked as playing a role in the uptake of water. The stimulatory effect of auxin may arise through the action of auxin on wall structure. There is some evidence to show that auxin is in some way concerned with the physical property of the cell wall. This possibility of turgor changes has been recognized, but the difficulties of measuring turgor pressures directly have hindered experimental work.

One interesting result obtained with the Apparent Osmotic Volume technique by McLaren and Mercer is that the Apparent Osmotic Volume of tissues after four days in both water and in auxin solutions varied inversely as the reciprocal of the osmotic pressure of the external solutions. This result, which refers to the final volume, does not necessarily indicate the changes which must have taken place as the volume increased with time. Unfortunately, comparative data for auxin-treated and control tissues have not been obtained over the same intervals. In experiments carried out at different times the Apparent Osmotic Volume for both auxin-treated and control tissue obeyed the Laws of Osmosis. These results, which are as yet tentative, strongly indicate that the importance of osmotic processes in controlling the uptake by tissues in water and auxin cannot be dismissed too lightly.

As we have seen, no satisfactory explanation has yet been proposed to explain the water relations of tissue slices in water and auxin solutions. Despite this failure, there seems no good reason for rejecting the Osmotic Theory, particularly in view of the tentative Apparent Osmotic Volume data. Further speculation would be fruitless. Experiment must supply the answer.

Turning next to the so-called physiological properties of the active uptake process in tissue slices in water and solutions, namely oxygen dependence, inhibition by respiratory inhibitors, high temperature coefficient and so on. It does not follow necessarily, because the wet weight of tissues is dependent upon the oxygen tension or is inhibited by respiratory inhibitors, that the increase in water absorption must be caused by an active uptake of water. Such a conclusion is far from warranted, especially since alternative explanations can readily be proposed. Correlations do not necessarily imply causation. There is room for speculation, and in an address of this kind one is allowed to speculate.

Little is known regarding the nature of the processes controlling the osmotic pressure of the cell sap in a tissue which is not deriving osmotic material from an external source. One important factor would be the balance between synthesis and breakdown. This would not be a single process, but a reflection of the overall metabolism of the tissue. In recent years tracer studies with carbon and nitrogen have demonstrated the extreme lability and interdependence of metabolic processes. In the steady state the metabolic processes proceed at certain uniform rates, continuing collectively, so to speak, to give the metabolism of the so-called resting cell. Synthesis and breakdown are balanced such that a particular level of solutes is present in the vacuolar sap. One might expect that any experimental treatment which influences almost any aspect of metabolism will eventually affect the concentration of solutes in the sap.

A few examples will be considered. Respiration is the key process in cell metabolism. Alteration in the respiration rate is likely to have a profound effect on many properties of the cell. One property which is dependent upon respiration is the organization and permeability characteristics of the cell membranes. Suppression of respiration either by inhibitors or a lowering of the oxygen tension is likely to increase the permeability of the cell membrane leading to a leakage of solutes from the vacuole. In this way the osmotic pressure would fall, resulting in the exosmosis of water. A loss of volume (or wet weight) arising in this way could be interpreted as due to the inhibition of an active water uptake process. It would be interesting to determine leakage rates of tissues treated for several days with cyanide or dinitrophenol.

The balance between synthesis and degradation, since enzymatic processes are involved, could well be temperature dependent. The high temperature coefficients sometimes observed for the water uptake by tissue slices in water could reflect a shift in the synthesis:degradation balance. At the best, a temperature coefficient is but a crude index of the metabolic activity of cells. To assume, solely on the grounds of temperature coefficients, the existence of an active water uptake process is unwarranted. A particular example of the possible importance in the shift from synthesis to breakdown may be the observation that the uptake of water by potato tissue slices has a high temperature coefficient, whereas the coefficient for carrot is low (Stiles, 1917). In potato which

has a high starch content, a shift in the starch:sugar balance may occur with high temperatures, leading to an increase in the osmotic pressure of the cell sap and to the increased uptake of water. Carrot is starch free, and has a low coefficient. In this example the high temperature coefficient for water uptake is apparent, not real. In a similar way, temperature may influence other cell processes via enzymatic processes and thereby water uptake.

The so-called physiological properties of water uptake do not provide unique evidence for an active uptake process. At the best, they probably reflect the general metabolic activity of the tissues. For this reason, and also since alternative metabolic explanations can be suggested, the conclusions based on the physiological properties should not be accepted too seriously.

There remains the difficulty of explaining the similar physiological properties of root pressure and exudation. Here again, factors such as temperature may influence the general metabolic activity rather than a unique water uptake process. The mechanism of root pressure is rather more complicated than the water relations of parenchyma cells. The essential feature of the process is the maintenance of an osmotic gradient between the xylem cells and the surrounding cells. The maintenance of the gradient through the secretion of solutes may well be the "active process" in root pressure. Water movement simply follows passively the solute movement. If such be the case, then the meaning of the physiological properties of root pressure and exudation becomes apparent.

Inhibition of the secretion of solute through a lowering of the temperature or through cyanide or azide would indirectly inhibit water movement. If water movement is the quantity measured, then it must have the physiological characteristics of a secretory process. We feel that this is likely to be the explanation of the active water uptake process in roots. Time does not permit a full discussion of the voluminous results on the problem, so the matter must be left here.

Also because of time, the water relations of oat coleoptiles, despite their obvious bearing on the active water uptake problem, cannot be discussed in any detail. However, a few general comments will be made—the first being to emphasize that the water relations of the coleoptile are much more closely associated with the problem of growth, whereas those of the tissue slices already discussed are essentially those of mature cells in which growth is absent or very limited. It is to be expected that the water relations of coleoptiles will be more complicated and more difficult to unravel. But as with the tissue slices, the explanation should be sought first in terms of changes in the osmotic quantities of the cells. Since the volumes of the cells increase to such an extent during the uptake of water, changes in the cell wall are likely to play a correspondingly greater part. Also since cell volumes increase greatly, the synthesis of cytoplasm and other cell components must influence the water uptake mechanism. The water relations problem in these tissues is probably an aspect of growth concerned with the synthesis of new cell wall material, cytoplasm, and osmotically active solutes.

In all probability, the physiological attributes of the water uptake in coleoptiles, as with the other tissues considered, reflect aspects of cell metabolism, but because of their immaturity, inhibitors, oxygen tension, temperature, and so on, are likely to have more spectacular effects. Inhibition or stimulation of water uptake by some external factor does not necessarily prove the existence of an active uptake process. The stimulatory effects of auxin must await a fuller understanding of the role of hormones in growth. So long as the possibility of auxin affecting the osmotic concentration of the cell sap via other metabolic processes or of auxin controlling wall growth and structure remains unproven, it would seem unwise to postulate that the auxin effect is via an active water uptake process.

In this address we have not been able to discuss all the anomalous results relating to the water relations of cells and tissues. Only some of the more important pieces of evidence on which the Active Uptake Theory has been based have been considered. We have shown that much of this evidence is open to serious criticism and should be rejected. In fact, when allowance is made for errors of technique and interpretation,

many of the so-called anomalous results have been shown to support, not to disprove, the Osmotic Theory. For the reasons which have been stressed already there seems little ground for postulating, from the evidence available, the existence of an active water uptake process in plant cells. Anomalies remain, and time alone will decide whether these reflect an active uptake process.

The Osmotic Theory: A Restatement.

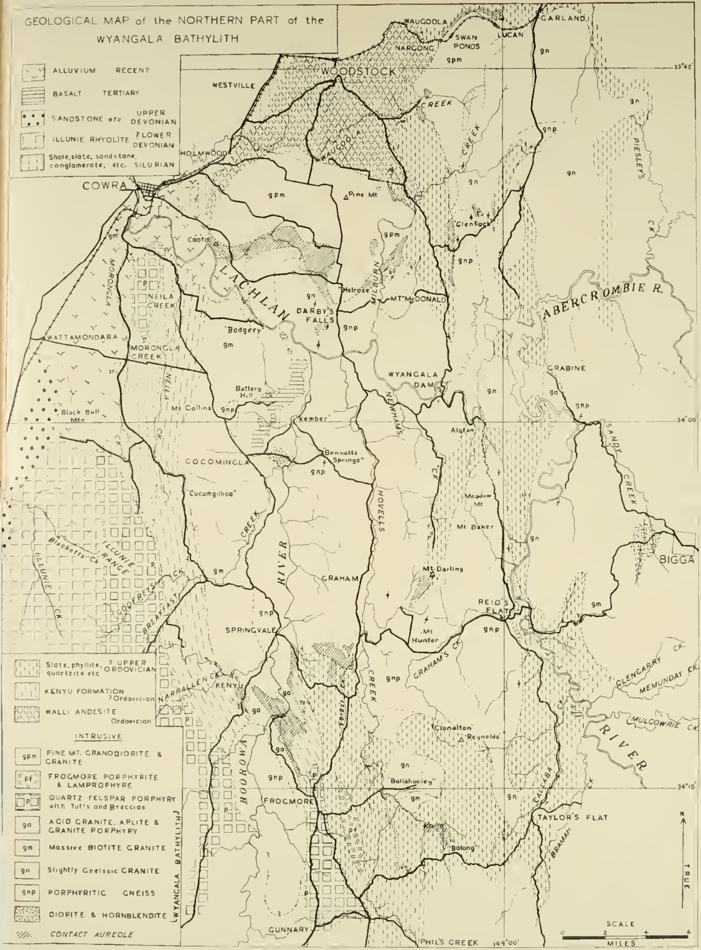
The number of examples showing that mature cells obey the laws of osmosis is so large that it is difficult not to accept the Osmotic Theory as an important and proven generalization concerning cell function. Admittedly the theory does not explain all known observations relating to the water relations of cells. We have seen in this address that many of the discrepancies between the behaviour expected on osmotic grounds and that observed are not contrary to the Osmotic Theory. Consequently, we seem justified in believing that other anomalous behaviour will also be explained within the framework of the Osmotic Theory.

One type of anomalous behaviour which will have a significant bearing on the future of the water relations problem is the small differences in volume observed between cells plasmolysed in isosmotic solutions of electrolytes and non-electrolytes. For example, the overall volume of the protoplast is greater in potassium nitrate solution than in sucrose or calcium chloride solutions. These differences appear to be associated with the cytoplasmic phase, and raise the problem of the water relations of the cytoplasm as opposed to that of the vacuole.

At the beginning of this address we mentioned that the Osmotic Theory was formulated to explain the water relations of the mature, not the meristematic, cell. Clearly some modification is required to cover all cells. Between the meristematic and the adult cell a whole range of cells occur in which a changing ratio of cytoplasm to vacuole is the most striking morphological characteristic. The overall water relations of the protoplast must be considered as the resultant of the water relations of vacuole and cytoplasm. The questions which must be answered in the future are "What are the similarities and differences between the water relations of these two systems?" and "Do active water uptake processes function in the cytoplasm?"

Ideally the Osmotic Theory requires the existence of a semipermeable membrane and a solution phase. This fact has been recognized from the beginning, and plenty of effort has gone into identifying these units with structures in the protoplast. With the mature cell no difficulties were experienced in identifying the solution phase with the vacuolar sap, but the position of the semipermeable membrane has always been a matter of controversy. Semipermeable properties have been ascribed to the tonoplast, to the external layers of the cytoplasm next to the cell wall, and to the cytoplasm as a whole. That the tonoplast has semipermeable properties was first clearly indicated by Höfler, who found that the vacuole-tonoplast system can remain intact and undergo volume changes without the enclosing layers of cytoplasm. More recently conclusive proof of the semipermeable nature of the tonoplast has been obtained by Mercer (1950) and Clark and Mercer (1955), who studied the water relations of both isolated vacuole-tonoplasts and isolated intact protoplasts. Both structures obeyed the Boyle van't Hoff Law almost exactly. Since the tonoplast-vacuole system functioned alone, it would seem certain that this system represented the osmometer of the intact protoplast. Similar conclusions relating to the position of the semipermeable barrier have been reached indirectly by other techniques. If the tonoplast-vacuole system is the osmometer of the protoplast, "what of the cytoplasm?".

Most of the evidence relating to the structure of cytoplasm supports the view that cytoplasm is a complicated sol:gel system. Furthermore, optical, differential centrifugation and other data show that the cytoplasm is differentiated into a more or less sol-like fraction containing the particulate structures such as the mitochondria and plastids. More recently electron microscope studies have confirmed this general structure. An electron micrograph of mature plasmolysed cells of beetroot, and turgid cells of *Nitella* obtained by Hodge, McLean and Mercer (1955) is shown in Plate A,



GEOLOGICAL MAP of the NORTHERN PART of the WYANGALA BATHYLITH
ALLUVIUM RECENT
BASALT TERTIARY
SANDSTONE etc UPPER DEVONIAN
ILLUNIE RHYOLITE ? LOWER DEVONIAN
Shale, slate, sandstone, conglomerate, etc. SILURIAN
Slate, phyllite, quartzite etc ? UPPER ORDOVICIAN
KENYU FORMATION ? Ordovician
WALLI ANDESITE Ordovician
INTRUSIVE
gpm PINE MT. GRANODIORITE & GRANITE
pf FROGMORE PORPHYRITE & LAMPROPHYRE
P QUARTZ FELSPAR PORPHYRY with Tuffs and Breccias
ga ACID GRANITE, APLITE & GRANITE PORPHYRY
gm Massive BIOTITE GRANITE
gn Slightly Gneissic GRANITE
gnp PORPHYRITIC GNEISS
DIORITE & HORNBLENDITE
CONTACT AUREOLE
WAUGOOLA
GARLAND
LUCAN
SWAN PONDS
NARGONG
WOODSTOCK
WESTVILLE
HOLMWOOD
COWRA
CREEK
PIESLEY'S CK
Pine Mt
Glen Rock
Coota
LACHLAN
Melrose
MILBURN
MT McDONALD
DARBY'S FALLS
ABERCROMBIE R.
NEILA CREEK
MORONGLA
WATTAMONDARA
"Bodgery"
MORONGLA CREEK
WYANGALA DAM
GRABINE
Battery Hill
Mt Collins
Black Bull Mtn
"Kember"
Alston
SANDY CREEK
"Bennetts Springs"
COCOMINGLA
"Cucumgilliça"
NEWHAMS CK
HOVELLS
Meadow Mt
Mt Baker
ILLUNIE RANGE
Blacketts Ck
ILLUNIE CK
GODFREYS CK
BREAKFAST CK
CREEK
RIVER
GRAHAM
Mt Darling
BIGGA
REID'S FLAT
Mt Hunter
SPRINGVALE
GRAHAM'S CK
GLENCARRY CK
MEMUNDAY CK
NARRALLEN CK
KENYU
CREEK
FOREST CK
MULCOWRIE CK
RIVER
'Clonalton'
Reynolds
BOOROWA
"Bellahooley"
FROGMORE
CALLABA CK
TAYLOR'S FLAT
BRANAH CK
"Bolong"
WYANGALA BATHYLITH
GUNNARY
PHIL'S CREEK
149°00'
33°45'
34°00'
34°15'
SCALE
MILES
TRUE N

fig. 1. The cytoplasm can be seen to consist of a deeply staining background material containing "empty areas" and mitochondria and plastids. Frequently the background material is further differentiated into linear aggregates as well as more or less homogeneous regions. There are no means available for telling just how far the structure of living cytoplasm corresponds with the fixed material as seen with the electron microscope. Since the structure is consistent with that postulated from other evidence, it seems reasonable to assume that the gross structure of the cytoplasm is similar to that seen with the electron microscope. Consequently it is assumed that the "empty areas" correspond to spaces previously occupied by solution and the densely staining material to the colloidal micelles of the solid phase of the living cytoplasm.

The electron microscope work shows beyond doubt the presence of two membranes in the adult plant cell (Plate A, fig. 2). This important observation supplies direct proof of the position and number of the main cell membranes, and clarifies an issue of fifty years' standing.

Finally, the electron microscope studies show that the particulate components, the mitochondria and plastids, consist of numerous, more or less well organized, densely staining lamellae embedded in a homogeneous, faintly granular material, and enclosed by a definite membrane.

Since cytoplasm has many of the properties of a gel system, it is useful at this point to look at the nature of the water relations of a fully imbibed protein gel. The water in a gel may be held by various electrostatic forces, either directly via hydrogen bonds and van der Waal forces, or indirectly through the osmotic influence of dissociable ions associated with the gel micelles. The force with which the water is held in the gel can be measured by the application of an external hydrostatic pressure, as is the case with a simple osmometer. Thus the imbibitional pressure so measured is analogous to an osmotic pressure. The distinction lies in the nature of the solutes responsible for the two types of pressure. In the simple osmometer the solutes are freely diffusible, whereas in the colloidal osmotic system the solute particles are non-diffusible, being held by the electrostatic charges of the colloidal micelles. The swelling of a colloid may be limited or non-limited, depending on the cohesive forces binding the colloidal micelles. Thus the water relations of a gel are determined by osmotic forces and the cohesive forces of the gel framework. The volume changes of a gel which occur during swelling will tend to obey the Laws of Osmosis, but deviations will occur, depending on pH, which influences the degree of dissociation of the groups providing the electrostatic bonds, and on temperature and the nature of the solutes present which influence the cohesive forces. Thus a gel is a particular kind of osmotic system in which the whole of the gel framework can be pictured as a semipermeable membrane, and the solution phase as being continuous throughout the membrane. This concept provides a useful basis for considering the water relations of the cytoplasm.

The well-known observations that the swelling of cytoplasm is a function of pH, the ionic composition of the external medium, and of temperature, show how closely the water relations of cytoplasm resemble those of a non-living gel. Despite these similarities, the possibilities of differences between the behaviour of the living and non-living systems should not be overlooked. In the absence of exact information about the water relations of cytoplasm, one cannot exclude the possibility of an active uptake process contributing to the water balance of cytoplasm. Another possibility, which we consider the more likely, is that the electrostatic and cohesive properties of cytoplasm are under the control of metabolism. Metabolism might affect the state of the cytoplasm via changes in the pH of the cytoplasmic solution, the concentration of solutes, the number of hydrogen bonds. If metabolism plays such a role, then the swelling of cytoplasm might differ considerably from the expected by analogy with a non-living gel. In this way the water relations might be found to have the physiological characteristics of a metabolic process, even though osmotic forces directly control the water balance.

The discussion of the water relations of cytoplasm would be incomplete if reference to the water relations of the particulate structures like the chloroplasts and mitochondria

were omitted. Evidence which is available indicates that these structures are highly organized lipoprotein systems of relatively low water content. Unpublished work (Farrant, Robertson and Wilkins, 1955) showed that the qualitative swelling of mitochondria varied with the osmotic pressure and ionic composition of the external solutions. In a recent paper (Mercer *et al.*, 1955), the swelling of chloroplasts isolated from *Nitella* was shown to obey the osmotic laws over a limited range of swelling, that is, they behaved like gels showing limited swelling because of their structure. In these chloroplasts swelling resulted from the volume changes of the interlamellar protinaceous material between the lamellae (Pl. A, fig. 2). The water balance was determined by the osmotic properties of the interlamellar material and the cohesive properties of the chloroplast. As with bulk cytoplasm, the imbibitional properties of both chloroplasts and mitochondria might be influenced by their metabolic activity. Be this as it may, the important point is that osmotic forces apparently control the water relations of the chloroplast, and possibly the mitochondria.

The evidence which we have discussed suggests that osmotic and cohesive forces determine the water relations of cytoplasm. At the present there does not appear to be any sound reason for assuming that non-osmotic water uptake processes play a contributory role. Thus I accept the point of view that the water relations of both the vacuole and cytoplasm can be interpreted on the basis of the Osmotic Theory.

In conclusion, the following picture of the protoplast is suggested as a working basis for interpreting cell water relations. The protoplast should be regarded as a two-phase system: a tonoplast-vacuole phase consisting of a solution phase enclosed by a semipermeable membrane, which behaves almost as a perfect osmometer; and a cytoplasmic phase, which behaves as a colloidal osmotic system, consisting of a micellar framework interspersed with a solution phase bounded externally by the relatively permeable cell membrane and internally by the tonoplast. Within the cytoplasm are the particulate structures which have the same type of osmo-regulatory mechanism as the bulk cytoplasm, but which, because of their highly ordered structure, show a more limited degree of swelling. Such a picture is not complete without a full appreciation of the osmo-regularity function of metabolism. Metabolism is suggested as the vehicle via which the osmotic pressure of the vacuolar sap and the imbibitional and cohesive forces of the cytoplasm are maintained.

This concept of the protoplast is applicable to all cells irrespective of age. The actual water relations of any particular tissue will depend on the extent to which the colloidal osmotic system contributes to the total water relations, and on the extent of metabolic control under the particular conditions. For this reason one can expect deviations from ideal osmotic behaviour, but these deviations do not imply that osmotic forces are not the controlling factors in cell water relations.

Finally, the dynamic side of water relations must be emphasized. Part of the confusion and difficulty experienced in interpreting water relations in terms of osmosis has been associated with the failure to appreciate the dynamic nature of the cell. Far too little attention has been given to the osmo-regulatory role of metabolism. Unfortunately, all too little is known regarding the way the osmotic properties of the cell are linked with other cell functions. The elucidation of this problem should provide a stimulus for future research in cell water relations.

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EXPLANATION OF PLATE A.

Fig. 1.—Thin section of Beetroot protoplast plasmolysed in 1·0M. glucose and fixed in 2% O_3O_4 at pH 7·2. Note the tonoplast (*t*) next to the vacuole (*v*) and the outer membrane (*OM*) next to the space (*s*) between the cell wall (*c.w.*) and the protoplast. Electron micrograph ×28,000.

Fig. 2.—A general view of the cytoplasmic layer in internodal cell of *Nitella* sp. Note the lamellar chloroplast (*ch*) enclosed by a membrane, several mitochondria (*m*) also enclosed by membranes, the lamellar structure (*cyt.l.*) and granular structure of the cytoplasm, and the tonoplast (*t*). Electron micrograph ×80,000.

The Honorary Treasurer, Dr. A. B. Walkom, presented the Balance Sheets for the year ended 28th February, 1955, duly signed by the Auditor, Mr. S. J. Rayment, F.C.A. (Aust.); and his motion that they be received and adopted was carried unanimously.

No nominations of other candidates having been received, the Chairman declared the following elections for the ensuing year to be duly made:

President: F. V. Mercer, B.Sc., Ph.D.

Members of Council: D. J. Lee, B.Sc.; F. V. Mercer, B.Sc., Ph.D.; S. Smith-White, B.Sc.Agr.; E. Le G. Troughton, C.M.Z.S., F.R.Z.S.; H. S. H. Wardlaw, D.Sc., F.R.A.C.I.; and A. R. Woodhill, D.Sc.Agr.

Auditor: S. J. Rayment, F.C.A. (Aust.).

A cordial vote of thanks to the retiring President was carried by acclamation.

LINNEAN SOCIETY OF NEW SOUTH WALES.

GENERAL ACCOUNT. Balance Sheet at 28th February, 1955.

LIABILITIES.	£ s. d.	£ s. d.
Capital—		
Amount received from Sir William Macleay during his lifetime ..	14,000 0 0	
Further sum bequeathed by his Will	6,000 0 0	
		20,000 0 0
Contingencies Reserve		10,741 11 4
Accumulated Funds		30,741 11 4
Bookbinding Account	905 1 11	
Income Account	875 7 2	
Suspense	6 0 9	
Current Liabilities		1,786 9 10
		£32,528 1 2

ASSETS.	£ s. d.	£ s. d.
Fixed Assets—		
Commonwealth Loans, at cost	15,048 10 0	
Debentures:		
Metropolitan Water, Sewerage and Drainage Board, at cost	1,694 7 6	
Science House (one-third share), at cost	14,835 4 4	
		31,578 1 10
Current Assets—		
Cash in hand	10 0 0	
Commercial Banking Company of Sydney, Ltd.	939 19 4	
		949 19 4
		£32,528 1 2

INCOME ACCOUNT. Year Ended 28th February, 1955.

	£ s. d.	£ s. d.
To Salary		604 3 4
„ Printing Proceedings	832 8 9	
„ Printing Reprints (Parts 1–4) ..	232 19 6	
„ Blocks	114 7 8	
		1,179 15 11
„ Insurance		14 6 4
„ Postage	69 14 10	
„ Petty Cash	23 7 11	
		93 2 9
„ Audit	16 16 0	
„ Printing and Stationery	85 0 6	
„ Expenses	37 10 1	
„ Cleaning	42 2 6	
„ Bank Expenses	2 11 9	
„ Library	31 2 9	
„ Blocks for colour postcards	73 17 2	
		289 0 9
„ Transfer to Bookbinding Account		196 4 7
„ Balance to 1955–56		875 7 2
		£3,252 0 10

	£ s. d.	£ s. d.
By Balance from 1953–54		111 8 3
„ Subscriptions: 1954–55	363 6 0	
Arrears	27 6 0	
In advance	14 14 0	
Associate	10 0	
		405 16 0
„ Entrance Fees		8 8 0
„ Interest		554 8 7
„ Science House		563 8 0
„ Sales		356 4 7
„ N.S.W. Government Grant		175 0 0
„ Fellowships Account (surplus income at 28th February, 1955, transferred)		320 18 9
„ Bank Expenses		4 15 2
„ Sale of Reprints (1953 and 1954)		586 2 6
„ Donations towards printing		65 12 0
„ Rent		2 4 0
„ Sale of sundries		2 15 0
„ Library Sales		92 10 0
„ Postcard Sales		2 10 0
		£3,252 0 10

AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1955, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1955, as shown by the books. Certificates of the investments have been inspected.

S. J. RAYMENT, Chartered Accountant (Aust.).

A. B. WALKOM,

LINNEAN SOCIETY OF NEW SOUTH WALES.

LINNEAN MACLEAY FELLOWSHIPS ACCOUNT.

Balance Sheet at 28th February, 1955.

LIABILITIES.	£ s. d.	ASSETS.	£ s. d.	£ s. d.
Accumulated Funds		Fixed Assets		
Amount bequeathed by Sir William Macleay	35,000 0 0	Commonwealth Loans, at cost ..	30,450 0 0	
Surplus Income Capitalized	20,097 1 7	Debentures:		
		Metropolitan Water, Sewerage and Drainage Board, at cost	16,306 14 9	
		Rural Bank of N.S.W., at cost ..	2,172 15 0	
		Loan on Mortgage	6,035 0 0	
				54,964 9 9
		Current Assets.		
		Commercial Banking Company of Sydney, Ltd.	128 5 3	
		Commonwealth Savings Bank ..	4 6 7	
				132 11 10
	£55,097 1 7			£55,097 1 7

INCOME ACCOUNT. Year Ended 28th February, 1955.

	£ s. d.		£ s. d.
To Salaries of Linnean Macleay Fellows	1,150 4 7	By Interest	1,920 18 9
„ Research and Field Expenses ..	75 0 0		
„ Balance, being Surplus Income transferred to General Account	320 18 9		
„ Capital Account	374 15 5		
	£1,920 18 9		£1,920 18 9

AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1955, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1955, as shown by the books. Certificates of the investments have been inspected.

A. B. Walkom,
Hon. Treasurer.

S. J. Rayment, Chartered Accountant (Aust.),
Auditor.

Sydney, 11th March, 1955. 1st March, 1955.